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MRID No. 444577-34

DATA EVALUATION RECORD
§ 72-3 - ACUTE LC₅₀ TEST WITH AN ESTUARINE/MARINE SHRIMP

1. **CHEMICAL:** Prohexadione Calcium PC Code No.: 112600
2. **TEST MATERIAL:** BAS 125 W Purity: 90.6%
3. **CITATION:**

Authors: W.C. Graves, J.P. Swigert, and C.M. Holmes
Title: BAS 125 W: A 96-Hour Static-Renewal Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
Study Completion Date: April 14, 1997
Laboratory: Wildlife International Ltd., Easton, MD
Sponsor: BASF Corporation, Agricultural Products, Research Triangle Park, NC
Laboratory Report ID: 147A-147
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4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist, Golder Associates Inc.

Signature: *Karl Bullock* Date: 7/7/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

Signature: *P. Kosalwat* Date: 7/7/98
5. **APPROVED BY:**

Signature: *R. Hanson* Date: 11/17/98
6. **STUDY PARAMETERS:**

Definitive Test Duration: 96 hours
Study Method: Static-renewal
Type of Concentrations: Mean Measured
7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements. The 96-hour LC₅₀ for mysid shrimp exposed to BAS 125 W was >125 ppm ai, which classifies this compound as practically non-toxic to *Mysidopsis bahia*. The NOEC was determined to be 125 ppm ai.

Results SynopsisLC₅₀: >125 ppm ai

95% C.I.: N/A

NOEC: 125 ppm ai

Probit Slope: N/A

8. ADEQUACY OF THE STUDY:**A. Classification:** Supplemental**B. Rationale:** The age of the test mysids was not reported.**C. Repairability:** Yes, if the mysids were ≤24 hours old.**9. GUIDELINE DEVIATIONS:** The age of the juvenile mysids used in the test was not reported; the guidelines require the use of ≤24 hour old mysids.**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS:****A. Test Organisms**

Guideline Criteria	Reported Information
<u>Species</u> Preferred species are <i>Mysidopsis bahia</i> , <i>Penaeus setiferus</i> , <i>P. duorarum</i> , <i>P. aztecus</i> and <i>Palaemonetes</i> sp.	<i>Mysidopsis bahia</i>
<u>Age</u> Juvenile, mysids should be ≤ 24 hours old	Juvenile, unspecified age
<u>Supplier</u>	In-house culture
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> minimum 10 days	Adult mysids were held at conditions similar to testing for 14 days.
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study and no feeding for 24 hours before the beginning of the test if organisms are over 0.5 g each.	Mysids were fed live brine shrimp nauplii daily during the exposure period to prevent cannibalism.
<u>Pretest Mortality</u> <3% mortality 48 hours prior to testing	Not reported

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Natural seawater pumped from the Indian River Inlet, DE, diluted to a salinity of approximately 20 ‰ with Wildlife International Ltd. well water.
Does water support test animals without observable signs of stress?	Yes
<u>Salinity</u> 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6‰	20 ‰

Guideline Criteria	Reported Information
<u>Water Temperature</u> Approx. 22 ± 1 °C	24.3 - 25.6°C
<u>pH</u> 8.0-8.3 for marine (steno-haline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	8.1 - 8.4
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 hrs and $\geq 40\%$ during 2 nd 48 hrs, Flow-through: $\geq 60\%$	$\geq 72\%$ throughout test
<u>Total Organic Carbon</u>	1.4 mg/l
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel 2. <u>Size:</u> 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. <u>Fill volume:</u> 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	1. Glass 2. 2-L beakers 3. 1500 mL
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	N/A
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	Test solutions were renewed on Day 2
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow-through: ≤ 1 g/L/day	1 mysid/15 mL

Guideline Criteria	Reported Information
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 h light, 8 h dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	None

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L with 30 shrimp, then no definitive test is required.	Yes; A range-finding test with a negative control and nominal concentrations of 7.5, 15, 30, 60, and 120 mg ai/L resulted in 0, 0, 0, 0, 0, and 20% mortality, respectively. One lethargic mysid was observed in the 120 mg ai/L treatment.
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	Negative control, 16, 26, 43, 72, and 120 mg ai/L.
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers	20 per level, 10 per replicate
<u>Test organisms randomly or impartially assigned to test vessels?</u>	Mysids were impartially distributed to each vessel.
<u>Biological observations made every 24 hours?</u>	Yes

Guideline Criteria	Reported Information
<u>Water Parameter Measurements</u> 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature and DO were measured at test initiation and termination as well as prior to and after each renewal (old and new solutions) in all test chambers and continuously in one negative control replicate pH was measured at test initiation and termination as well as prior to and after each renewal (old and new solutions) in alternate replicate test chambers.
<u>Chemical Analysis</u> needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Samples collected at test initiation and termination from each test chamber as well as at 48 hours from replicate A of the "old" solutions and replicate B of the "new" solutions were analyzed by HPLC.

12. REPORTED RESULTS:

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	Average: 104-109%
<u>Control Mortality</u> Not more than 10% of control organisms may die or show abnormal behavior.	0% mortality in the control group
Raw data included?	Yes
Signs of toxicity (if any) were described?	No sublethal signs of test material toxicity were observed.

Mortality

Concentration (mg ai/L)		Number of Shrimp	Cumulative Number Dead			
Nominal	Mean Measured		Hour of Study			
			24	48	72	96
Control	<0.05	20	0	0	0	0
16	17	20	0	0	0	0
26	27	20	0	1	1	1
43	47	20	0	1	1	1
72	77	20	0	1	1	1
120	125	20	0	0	0	0

Other Significant Results:**B. Statistical Results**

Method: Visual observation

96-hr LC₅₀: >125 mg ai/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 125 mg ai/L

13. VERIFICATION OF STATISTICAL RESULTS:

Parameter	Result
Binomial Test LC ₅₀ (C.I.)	N/A
Moving Average Angle LC ₅₀ (95% C.I.)	N/A
Probit LC ₅₀ (95% C.I.)	N/A
Probit Slope	N/A
NOEC	125 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound but does not meet the guideline requirements for an acute toxicity test using *Mysidopsis bahia*. The age of the test mysids was not reported. Based on mean measured concentrations, the 96-hour LC₅₀ was >125 ppm ai, which classifies BAS 125 W as practically non-toxic to mysid shrimp. The NOEC was 125 ppm ai. This study is classified as **Supplemental**.